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Review

Hearing Impairment: A Panoply of Genes and Functions

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Research in the genetics of hearing and deafness has evolved rapidly over the past years, providing the molecular foundation for different aspects of the mechanism of hearing. Considered to be the most common sensory disorder, hearing impairment is genetically heterogeneous. The multitude of genes affected encode proteins associated with many different functions, encompassing overarching areas of research. These include, but are not limited to, developmental biology, cell biology, physiology, and neurobiology. In this review, we discuss the broad categories of genes involved in hearing and deafness. Particular attention is paid to a subgroup of genes associated with inner ear gene regulation, fluid homeostasis, junctional complex and tight junctions, synaptic transmission, and auditory pathways. Overall, studies in genetics have provided research scientists and clinicians with insight regarding practical implications for the hearing impaired, while heralding hope for future development of therapeutics.

Causes of Hearing Loss

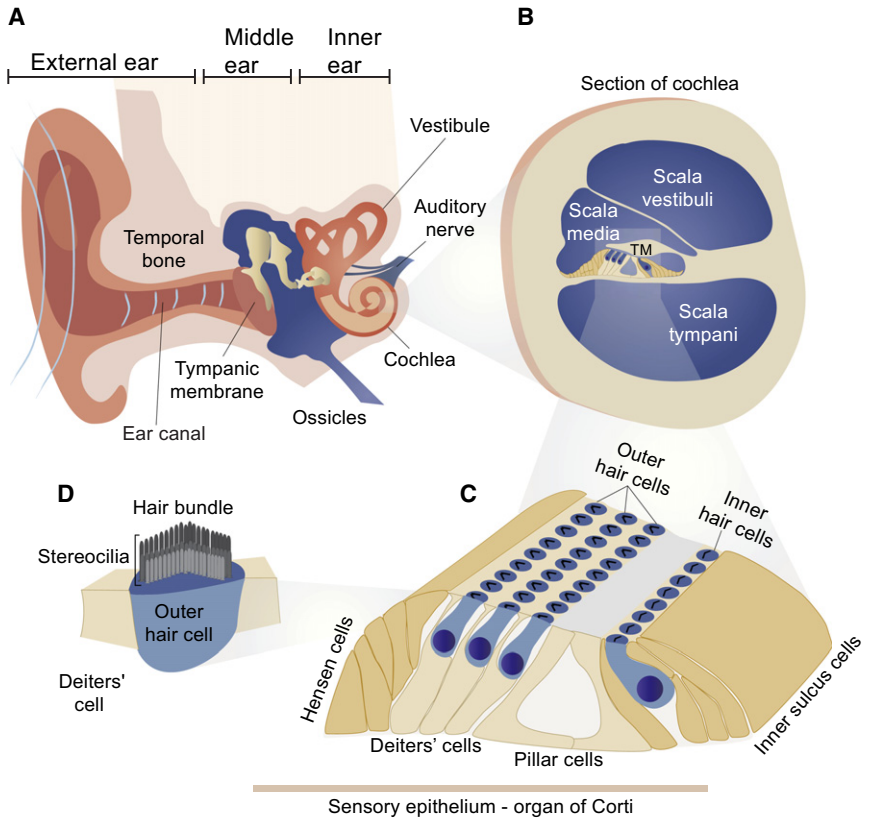
Similar to other sensory loss, hearing impairment has a wide spectrum of etiologies originating from both environmental and genetic factors. Prolonged exposure to high intensity sound poses high risk for auditory function and can lower hearing thresholds. Acoustic trauma, as a result of a sudden loud noise, can lead to temporary and/or permanent hearing impairment. Among environmental factors, different viral infections, as well as neonatal anoxia and hyperbilirubinemia, can also cause permanent hearing defects. Long-term augmentation of ototoxic drugs such as aminoglycoside and gentamicin antibiotics has an adverse effect on the auditory system and accounts for hearing defects (Yorgason et al., 2006). Unlike the genetics factors dictated by hereditary information, some of the environmental factors can be reduced or prevented by raising awareness for appropriate protection.

Genetic insults contributing to hearing defects poses greater challenges. The clinical heterogeneity of hearing loss is characterized by common classifications based on several parameters such as onset, severity, and the presence of additional clinical manifestations other than deafness. Hearing loss that occurs prior to speech acquisition is termed prelingual deafness, either congenital or appearing after birth. A hearing disability that appears early in childhood can have a major consequence on language acquisition. Age-related hearing loss (ARHL) affects the elderly population with high prevalence, and its appearance and progression is influenced by both genetic and environmental factors (Cruickshanks et al., 1998; Gates et al., 1999). About 60% of the population over the age of 65 suffers from different degrees of hearing loss, with a decline in sensitivity to sound, accompanied with reduced speech perception. Hearing loss is also categorized based on the frequency loss and the severity of hearing thresholds. High tone loss refers to reduced sensitivity of high-frequency acoustic stimulus, as opposed to low tone loss for the low frequencies. The terms profound, mild, and moderate describe the descending order of different severity levels of hearing impairments. When hearing loss is the only apparent abnormality, it is referred to as nonsyndromic hearing loss (NSHL). In other cases hearing loss occurs along with a variety of other malformations and thus is designated as syndromic hearing loss (SHL).

Overview of the Hearing Mechanism

The auditory system bears one of the most intricate mechanisms of sensation ability in humans. The inner ear, a fluid-filled organ, is responsible for transforming the mechanical energy of the sound waves into electrical stimuli, which will eventually be translated in the brain. Anatomically, the inner ear is divided into the auditory and vestibular systems. While the auditory system is responsible for sound sensation, the vestibular system is responsible for three-dimensional orientation and gravity perception. The similarities between these two systems often lead to balance disorders in hearing impaired individuals (Gresty and Brookes, 1997). The auditory system is composed of a snail-shaped cochlea. The cochlea is a fluid-filled tube coiled in a spiral shape around the modiolus (Figure 1). Upon viewing a longitudinal cross-section, the cochlear canal is divided into three compartments (scalae). The scala media filled with endolymph lies between two larger perilymphatic filled compartments, the scala vestibuli and scala tympani.

The scala media contains the cochlear sensory epithelium, the organ of Corti, which sits on top of the basilar membrane (Corti, 1851). The organ of Corti contains specialized sensory cells, known as hair cells, arranged in three rows of outer hair cells (OHCs) and one row of inner hair cells (IHC). The tectorial membrane, which sits on top of the organ of Corti, is an extracellular auxiliary structure contributing to hair-cell excitation (Lukashkin et al., 2010). Sound-induced mechanical vibration of the middle ear is transmitted to the cochlea, generating movements of its associated fluids (Lawrence et al., 1961). As a consequence, deflection of the basilar membrane activates the sensory cells that transduce the mechanical stimulation



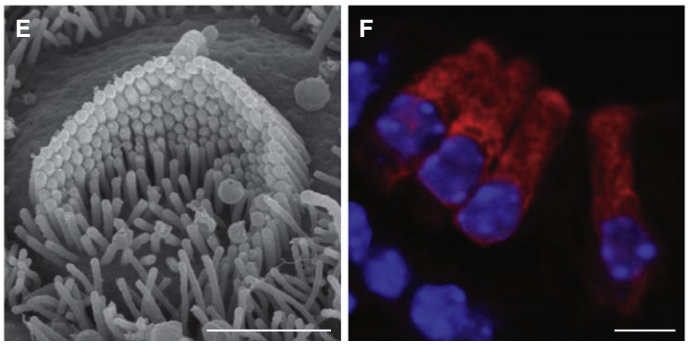


Figure 1. Schematic Illustration of the Mammalian Inner Ear

(A) The ear is composed of the external, middle, and inner ear. The cochlea, responsible for hearing, and the vestibule, responsible for balance, make up the inner ear.

(B) A cross-section of the cochlear duct reveals the scala media, scala tympani and scala vestibuli that are filled with fluids, as well as the tectorial membrane (TM) over the organ of Corti.

(C) An enlargement of the organ of Corti, the cochlear sensory epithelium, showing three rows of OHCs and one row of IHCs, flanked by various types of supporting cells.

(D) The hair bundle of an OHC showing the actin-based stereocilia organized in a staircase pattern.

(E) Scanning electron microscopy (SEM) image showing the hair bundle of an OHC of a mouse, analogous to the scheme in (D) (our unpublished data).

(F) Immunofluorescence confocal images of IHCs and OHCs of a mouse, with expression of myosin VI (red) in the cytoplasm and the nucleus marked by DAPI (blue), analogous to the scheme in (C) (our unpublished data).

(A–D) Adapted from Dror and Avraham (2009).

into electrical signal. The number of cochlear turns, combined with the graded length and stiffness of the basilar membrane along the length of the cochlea, contributes to the determination of the audible range of frequencies (Manoussaki et al., 2006).

The cochlear hair cells have a substantial role in translating mechanical forces evoked by sound into an electrical signal. The apical surface of each hair cell contains protrusions of actin-rich filaments known as stereocilia, which play a pivotal role in this mechanism. These membrane-bound filaments form a typical staircase arrangement, stabilized by a rich network of interconnections. Most significant, a tip link is present between the tops of stereocilia in the upper row of the hair bundle to the tips of stereocilia on the next lower row (Kazmierczak et al., 2007; Pickles et al., 1984). Upon mechanical stimulation that force deflections of the hair bundles, the tip links trigger the opening of the mechanoelectrical transduction (MET) channels that are located at stereocilia tips across the bundle. The synchronized opening of the MET channels depolarizes the cells and initiates electrical signals to the auditory nerve. The hair bundles of the cochlear hair cells face the scala media immersed in its fluid, the endolymph. Unlike other physiological fluids in the human body, the endolymph has a unique electrolyte composition with high potassium (K^+) and low sodium (Na^+) concentrations (Wangemann and Schacht, 1996). When hair cells are mechanically excited, an influx of potassium (K^+) and calcium (Ca^{2+}) ions depolarize the cell and trigger the release of neurotransmitter at the basal pole of the hair cell (Dallos, 1996).

The afferent auditory pathway connects the sensory machinery of the cochlea to the brain, paving the route for propagation of neuronal electrical signals evoked by an acoustic stimulus. The apical side of cochlear hair cells is responsible for their mechanosensory role mediated by hair bundles, whereas the basolateral side of the cell is responsible for synaptic transmission. Thus, IHCs also function as presynaptic terminals, by coding acoustic signals to neurotransmitter release onto auditory afferent nerve fibers (Fuchs, 2005). Specialized ribbon synapses located at presynaptic active zones of IHCs are tethered by synaptic vesicles and are sufficient for their precise temporal release in response to sound (Glowatzki et al., 2008). IHCs serve as the major acoustic sensors, whereas the OHCs increase amplification sensitivity and frequency selectivity of the cochlea (Dallos, 1992). Each IHC is innervated by more than 15 afferent sensory

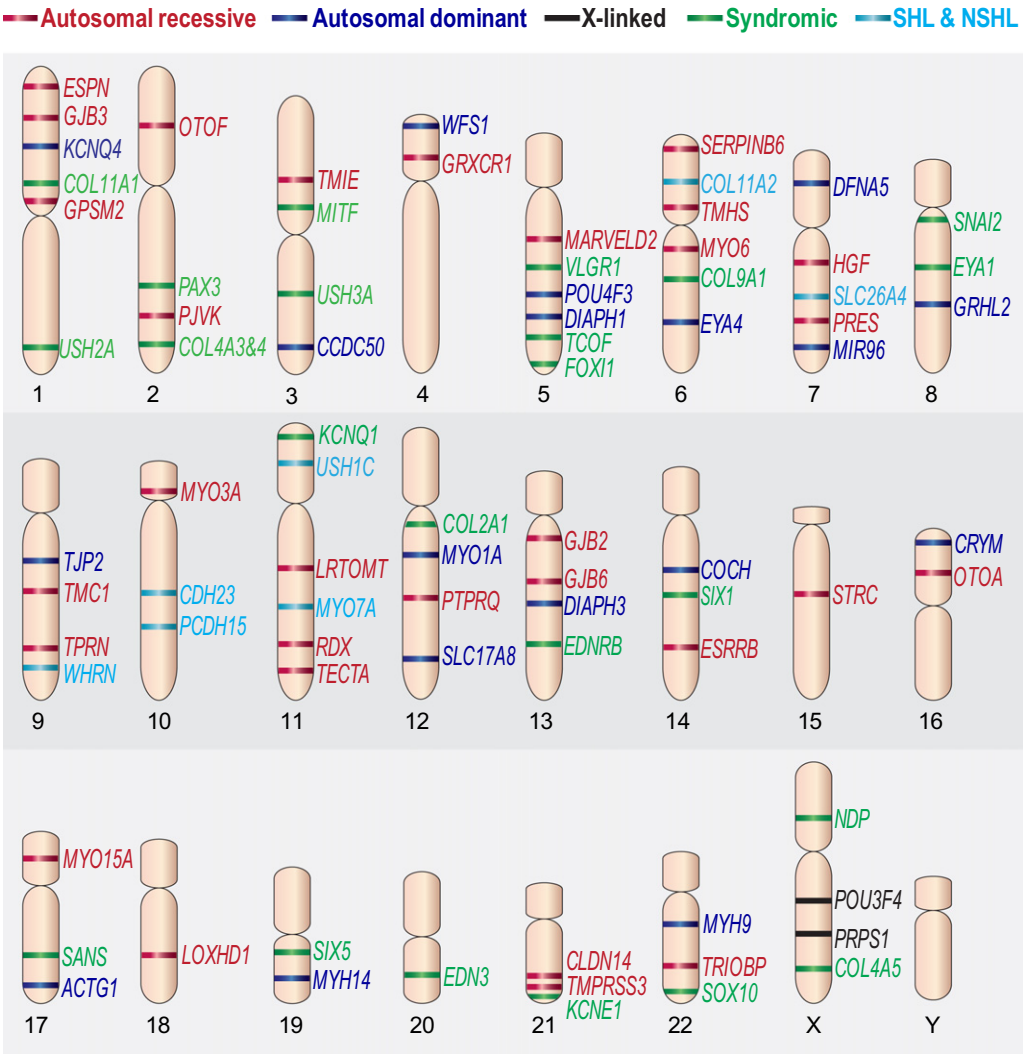


Figure 2. The Chromosomal Location of Genes with Mutations Causing Hearing Impairment
The genes are classified as nonsyndromic autosomal recessive (red), nonsyndromic autosomal dominant (blue), x-linked (black), syndromic (green), and genes that are associated with both syndromic and nonsyndromic hearing loss (light blue). Data was taken from the Hereditary Hearing Loss Homepage.

neurons, providing efficient parallel channels for transmission of an acoustic stimulus to the central nervous system (Rubel and Fritzsch, 2002). The primary electrical signal initiated by the IHCs is processed in the spiral ganglia, the auditory nerve, and integrated in the afferent auditory pathway. Further downstream to the auditory nerve, the auditory pathway contains four major intermediate stations, including the cochlear nuclei, superior olive, inferior colliculus, and medial geniculate body (Webster, 1992). In the brain, the collected auditory inputs are decoded and analyzed within the auditory cortex in the temporal lobe. The cochlear hair cells are arranged in a tonotopic gradient that enables acquisition of sensory transduction of the audible range of frequencies. This organization is characterized by a gradient along the length of the cochlea, sensing high frequencies at the base and low frequencies at the apex (Romand, 1997). Likewise, further communication of acoustic signals with the brain is tonotopically preserved within the different levels of the auditory pathway (Rubel and Fritzsch, 2002). This high selectivity and sensitivity for frequency specific signals provides us with the ability of orchestrated perception to decipher between a rich spectrum of sounds.

Inroads into the Genetics of Hearing and Deafness

Given the complexity of the hearing mechanism, it should come as no surprise that a panoply of genes have been discovered to be involved in hearing loss. To date, more than 50 genes and 80 additional loci have been linked to various degrees of hearing impairment (Figure 2). Taking advantage of standardized nomenclature, a common classification of the loci and genes for hearing impairment has been established (HUGO Gene Nomenclature Committee, http://www.genenames.org/). Depending on the inheritance mode, the nonsyndromic genes or loci are classified accordingly: DFNA (dominant), DFNB (recessive), DFNX (x-linked), DFNY (y-linked), and DFNM (modifier). Additional

specific symbols are used for different forms of hearing loss including otosclerosis (OTSC), auditory neuropathy (AUNA), and mitochondrial (MRTNR, MTTS) genes. For each locus, the relevant symbol is depicted with a number next to it, designated by the chronological order of its discovery. Routinely updated, the Hereditary Hearing Loss Homepage (http://hereditaryhearingloss.org/) provides an open and reliable resource for all listed genes and loci. Well-established genetic studies from the past years have highlighted the pathophysiologies underlying mutations in many of these genes (Dror and Avraham, 2009). Several studies have successfully integrated groups of proteins encoded by these genes into common pathways of inner ear function, providing explanations for the similar phenotypes of affected individuals carrying mutations in different genes of the same network. One such example is the Usher network of proteins, with mutations in nine different genes underlying this most common syndrome of deafness and blindness (Saihan et al., 2009) Nevertheless, human variants of the same gene may result in clinical heterogeneity. For example, mutations in the *SLC26A4* gene are linked with either NSHL, DFNB4, or a syndromic form known as Pendred's syndrome (PS) with enlargement of the thyroid gland (Pera et al., 2008).

The attempt to identify genes for hearing impairment by conventional methods has led to great success over the past two decades. Linkage analysis with microsatellite markers has been used widely, allowing the chromosomal location of deafness genes to be mapped in families all over the world. Once the linkage region was elucidated, in the most recent years, mutation analysis by Sanger sequencing often led to the identification of the causative mutation. However, despite the great contribution of linkage analysis methods, many deafness genes remain to be elucidated. A long list of human loci linked with hearing impairment is still pending for further gene discovery (Hereditary Hearing Loss Homepage). Furthermore, complex mutations such as duplications of an entire gene within a detective locus could not be assessed by standard sequencing approaches. For example, taking advantage of array comparative genomic hybridization (array CGH), geneticists can track chromosomal imbalances. Utilizing this platform, a tandem genomic duplication of the *TJP2* gene was recently identified to be responsible for progressive NSHL in DFNA51 individuals (Walsh et al., 2010a). Both the *DFNB79* and *DFNB82* genes, encoding taperin and GPSM2, respectively, were identified by targeted genome capture, combined with massively parallel sequencing (Rehman et al., 2010; Walsh et al., 2010b). With the development of advanced new technologies such as massively parallel sequencing, it is expected that more genes will be added to the list of human genes for hearing impairment in a relatively short period.

Animal models have provided an invaluable tool for studying advanced hearing mechanisms in a way that could not have been achieved only by human studies. A wide array of organisms, including zebrafish, chick, and mouse, have complemented the human genetics field with an in-depth understanding of protein function. Among the models, the striking similarity between human and mouse inner ear structure and function has defined the mouse as a prominent animal model for human deafness. The ease of gene overexpression, depletion, and targeted mutagenesis has enabled researchers to create reliable animal models for genetic forms of hearing loss to mimic the corresponding mutation in humans. Once a novel human deafness gene is discovered, the generation of an animal model is optimal for studying its function. Thus, in parallel to gene discovery in human families, a long list of mouse models for hearing loss have been established (Leibovici et al., 2008). This tight crosstalk between human and mouse genetics also benefits in the other directions, from mouse to human (Brown et al., 2008). Mutants that have arisen due to spontaneous mutations and chemically induced mutants generated by *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis have led to the discovery of new deafness genes in mice and subsequent discovery of their human orthologs (Brown et al., 2009). For example, identification of a recessive mutation in the *Loxhd1* gene of the *samba* deaf ENU mice led to the discovery of its human ortholog *LOXHD1* within the previously mapped DFNB77 locus responsible for autosomal recessive NSHL (Grillet et al., 2009). This phenotype-driven approach has not only enriched the list of known deafness genes, but also enabled scientists to further study the pathophysiology underlying different mutations. The availability of state-of-the-art scientific tools, including in vivo studies on animal models, has opened a new venue for understanding the complex mechanisms of proteins in the wide context of the auditory network.

Molecular Pathways and Genes Involved in Hearing Loss

Gene discovery in humans and protein characterization in animal models have revealed numerous molecular pathways in the inner ear. These include but are not limited to gene regulation, fluid homeostasis, mechanotransduction, and structure (Figure 3).

Gene regulation plays an essential role in development. It is therefore not surprising that numerous transcription factors, including *EYA4*, *POU3F4*, *POU4F3*, *TFCP2L3*, and *ESRRB*, have been linked with hearing loss. Recently, a mutation in the microRNA miR-96 was also implicated in progressive hearing loss in humans, introducing the first microRNA deafness gene (Mencía et al., 2009). miR-96 resides within a cluster of three miRNAs, while the other two, miR-183 and miR-182, are also expressed in the inner ear and participate in the regulation of gene expression (Figure 4; Weston et al., 2006).

A wide number of genes encoding different transporters and channels are highly expressed in the ear and participate in maintaining the unique fluid homeostasis (Lang et al., 2007). The critical nature of maintaining appropriate fluid homeostasis is highlighted by evidence that mutations in genes such as the solute carrier transporter gene *SLC26A4* (pendrin) lead to prelingual deafness. Constant secretion of potassium into the endolymph and generation of endochlear potential is also paramount for inner ear physiology and requires potassium recycling machinery (Zdebik et al., 2009), with a network of connexin gap junction proteins suggested to support this process.

The compartmentalization of the two distinct extracellular fluids of the inner ear, endolymph and perilymph, requires a network of epithelia to establish a tight junctional barrier surrounding the scala media (endolymph). In this regard, a group

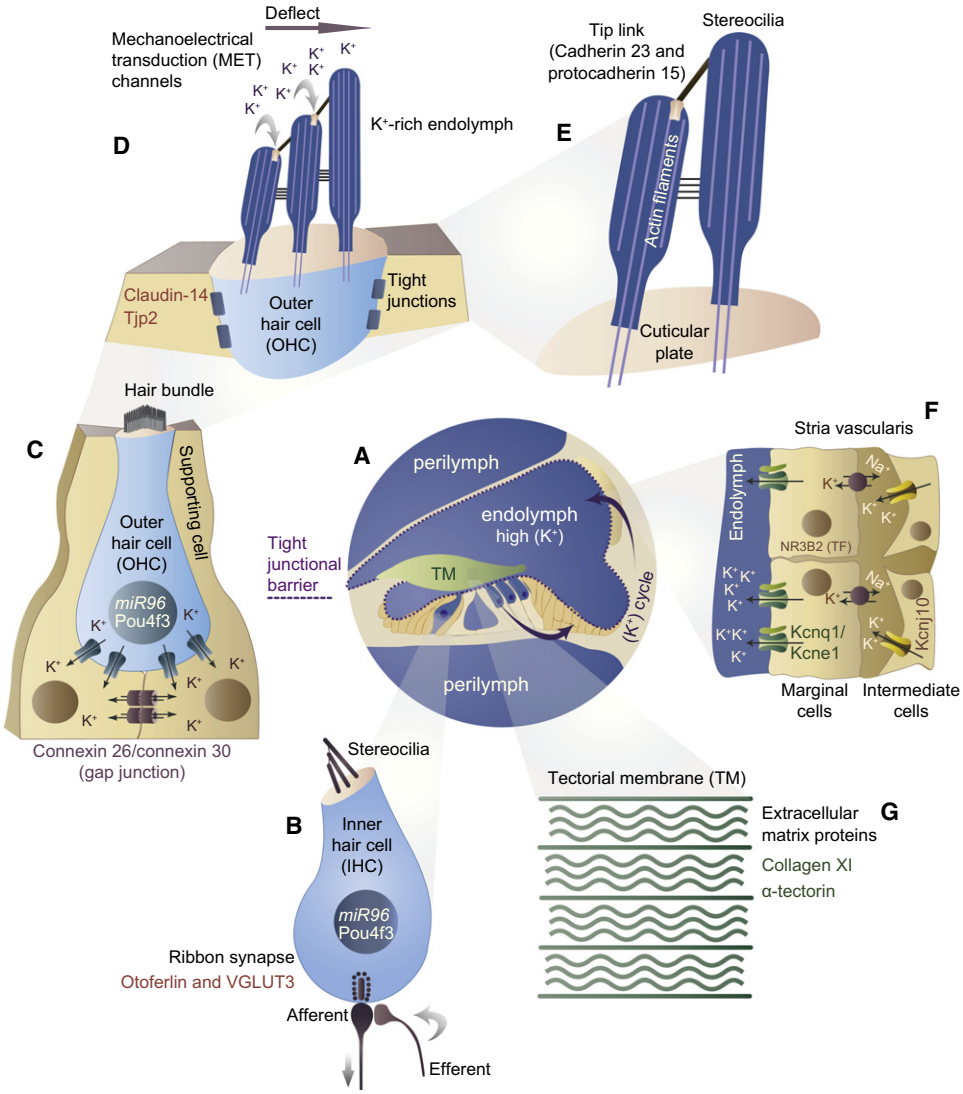


Figure 3. Schematic Illustration of Components of Hearing Mechanisms, Highlighting Genes Underlying Hearing Loss in Humans and Mice

(A) To support the unique endolymphatic fluid composition, a tight junctional barrier (dashed purple line) between epithelial cells of the scala media (endolymph) is established by different junctional complex proteins. This network of epithelia prevents leakage of the high potassium concentration that is constantly secreted into the endolymph by the (K^+) recycling machinery.

(B) At the lower part of the inner hair cell within the basal pole, a dense structure of ribbon synapse is tethered by a pool of synaptic vesicles ready to be released. A mechanosensory hair bundle on the apical side of the cells responds to an acoustic stimulus that depolarizes the cell, triggering secretion of synaptic vesicles.

(C) The cochlear hair cells are neighboring with supporting cells that establish a route for potassium ion propagation as part of its recycling machinery. Efflux of potassium outside the cell is supported by (K^+) channels and is crucial in order to bring the cell back to the excitatory condition.

(D) The apical surface of the hair cells is immersed within the endolympahtic fluids and is thus sensitive to its mechanical movements that are evoked by sound. Deflection of the hair bundle stretches tip links between stereocilia and triggers the opening of the MET channels, followed by influx of potassium ions that depolarize the cells. The tight junction barrier between hair cells and supporting cells prevents ion leakage from the K^+ rich endolymph and maintains selective paracellular transport.

(E) The tip links between actin-rich stereocilia are assembled with two adhering proteins. Other lateral links between sterocilia are essential for the cohesion and stability of the hair bundle.

(F) The stria vasularis, located at the lateral wall of the scala media, is essential for the secretion of (K^+) into the endolymph and for maintaining its associated endocochlear potential. The stria marginal and intermediate cells are rich with transporters and channels that support and maintain the homeostasis of the endolymph.

(G) The tectorial membrane (TM) is an auxiliary structure composed of extracellular matrix and contributes to hair cell excitation. Its integrity is highly depended on the temporal and spatial secretion of it associated proteins.

Adapted from Dror and Avraham (2009).

of genes encoding tight junction proteins, including *CLDN14*, *TRIC*, and *TJP2*, participates in the formation of the mechanical barrier between epithelial cells in the inner ear. These genes have also been implicated in different forms of hearing impairment and their functional significance in hearing is outlined, including barrier formation, cell polarization, and signal transduction.

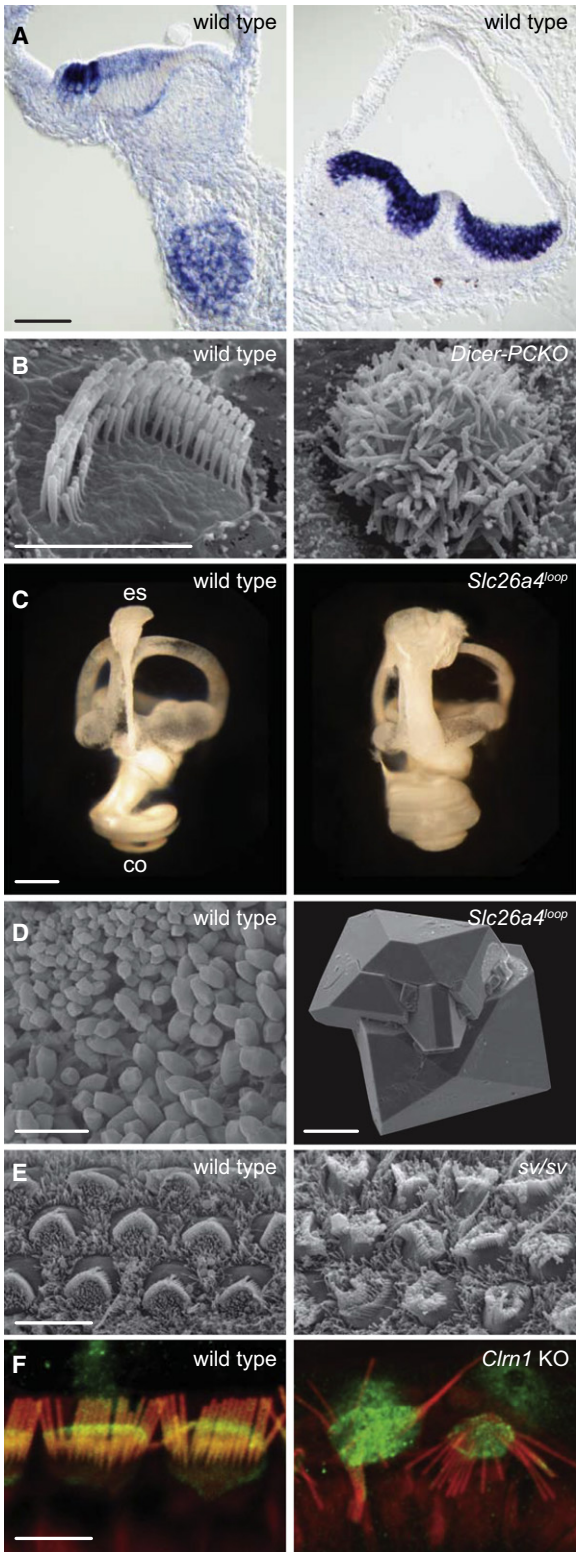


Figure 4. A Sampling of Expression in the Inner Ear and Morphological Defects in Deaf Mouse Mutants

(A) RNA in situ hybridization of microRNA-182 shows strong expression in the inner and outer hair cells of the cochlea (left panel), as well as in vestibular hair cells in the sensory epithelium of the crista (right panel). microRNA-182 is also expressed in the spiral ganglia of the cochlea (our unpublished data).

(B) A *Dicer-PCKO* mouse at p38, lacking microRNAs in the hair cells, has rounded hair bundles as opposed to the V-shape hair bundle of a wild-type mouse that shows an organized staircase structure of sterocilia, demonstrated by SEM (Friedman et al., 2009).

(C) Pendrin defects in the *Slc26a4*^{loop} mutant leads to hydrops of the endolymphatic labyrinth, demonstrated by paint filled inner ears. A prominent bulged cochlea (co) and endolymphatic sac (es) and duct is observed in the mutant (our unpublished data).

(D) Impaired transport activity of pendrin in the *Slc26a4*^{loop} mutant leads to formation of giant calcium oxalate mineralized bodies in the inner ear (Dror et al., 2010).

(E) Snell's waltzer (*sv*^{Myo6}) mice are null for the myosin VI protein and their cochleas lacks the normal cohesion and polarity of hair bundles, along with inconsistent direction of sterocilia protrusions (our unpublished data).

(F) The Usher syndrome III mouse model (USH3A) depleted of *Clrn1* shows severely disorganized hair bundles of inner hair cells with fused actin stereocilia (red) and altered size and shape of cuticular plate, detected by immunostaining against myosin VI (green) (our unpublished data).

Scale bars: In (A), 50 μm; in (B), 2 μm; in (C), 1 mm; in (D), 2 μm, 100 μm; in (E), 5 μm; (F), 2 μm.

The inner ear is responsible for transforming the mechanical energy of the sound waves into electrical stimuli, and its function relies critically on the integrity of the extracellular matrix of the tectorial membrane and the basilar membrane in order to achieve proper mechanical stimulation of the cochlear sensory cells. This process depends upon on the appropriate temporal and spatial expression patterns of the participating matrix proteins (Richardson et al., 2008). Accordingly, several extracellular matrix proteins encoded by *TECTA* (α-tectorin), *COL11A2 (*collagen, type XI, alpha 2), *COCH* (cochlin), *OTOA* (otoancorin), and *STRC* (stereocilin) have been associated with different forms of hearing impairment.

The inner ear also expresses another prominent group of genes belonging to the myosin family of motor proteins, including *MYO1A*, *MYO3A*, *MYO6*, *MYO7A*, *MYO15A*, and *MYH9* (Petit and Richardson, 2009). Hair cell-specific myosins were shown to have a crucial role in hair bundle organization and function. Human mutations of these myosins are associated with NSHL, while *MYO7A* mutations can also lead to a syndromic form of blindness and deafness known as Usher syndrome. Human mutations in several additional genes have also been linked to familial cases of Usher syndrome, while their encoded proteins are essential for the morphogenesis and cohesion of hair bundles of cochlear hair cells (Saihan et al., 2009). Four of these genes, *CDH23*, *PCDH15*, *USH2A*, and *VLGR1*, encode adhesion proteins; three genes, *WHRN*, *USH1C*, and *SANS*, encode scaffolding proteins; and the *USH3A* gene encodes an integral protein. The cell-cell adhesion proteins cadherin 23 (*CDH23*) and protocadherin 15 (*PCDH15*) form the tip link between adjacent stereocilia (Kazmierczak et al., 2007) and support the mechanical tension of the hair bundle and its mechanotransduction, with mutations in these genes causing NSHL or Usher syndrome (Ahmed et al., 2003; Bork et al., 2001). More details on these genes and their roles in deafness have been reviewed in detail elsewhere (Gillespie and Müller, 2009).

Finally, the normal function of the auditory system also depends critically on its ability to transduce mechanical stimuli into an electrical signal that appropriately propagates from the cochlea to the brain. Mutations in genes that are critical to this

synaptic transmission process, such as *SLC17A8* (VGLUT3) and *OTOF* (otoferlin), can lead to deafness, including a relatively rare hearing disorder, auditory neuropathy, associated with improper functioning of the auditory nerve.

In the current review we have focused on a subgroup of genes encoding proteins associated with different aspects of auditory function, including gene regulation, fluid homeostasis, junctional complex and tight junctions, synaptic transmission, and the auditory pathway. Individual genes and pathways have been chosen as a way to highlight the types of processes critical for proper auditory function, and illustrates how alterations in these gene products can lead to hearing impairment.

Gene Regulation

Temporal and spatial regulation of gene expression is fundamental for development, cellular proliferation and differentiation, morphogenesis, and drives the specific function of different cells and tissues (Latchman, 2007). A large group of regulatory proteins, including transcription factors (TFs) that bind to specific DNA sequences, regulate the precise transcription of a gene into RNA molecules and are therefore critically involved in many aspects of cellular development and function. Not surprisingly, mutations in TF genes have been detected in patients with a variety of different hereditary clinical conditions. Among the long list of deafness genes, several TFs harbor mutations that lead to multiple forms of hearing loss. Two of these, *POU3F4* and *POU4F3*, belong to the family of POU homeodomain *trans*-regulatory factors. In addition to their homeodomain, a second DNA-binding region, the POU domain, enhances the specificity to certain DNA sequences (Herr et al., 1988). *POU3F4* underlies X-linked DFNX2 (de Kok et al., 1995). Targeted mutagenesis of *Pou3f4* in mice leads to developmental defects of the auditory system (Phippard et al., 1999). Mutations in *POU4F3* underlie progressive hearing loss in humans (DFNA15) and affect its transcription activity and nuclear localization in the inner ear (Collin et al., 2008a; Vahava et al., 1998; Weiss et al., 2003). *Pou4f3* knockout mice are deaf due to the loss of cochlear hair cells (Erkman et al., 1996). In the mouse inner ear, *Pou4f3* is highly expressed in sensory hair cell nuclei and is essential for their maturation and survival (Xiang et al., 1998). Transcription profiling of *Pou4f3* mutant mice identified several downstream targets of *Pou4f3* in the inner ear including *Gfi1* and *Lhx3* (Hertzano et al., 2004). Recent work has demonstrated the potential treatment of antiapoptotic factors that mediate the survival of auditory hair cells derived from the *Pou4f3* mutant (Atar and Avraham, 2010). The EYA4 transcriptional activator has an important role during the maturation of the organ of Corti, and mutations cause late-onset deafness in humans (DFNA10) (Wayne et al., 2001). Mutations in another TF, *TFCP2L3*, a mammalian ortholog of the *Drosophila* gene *grainyhead*, leads to progressive hearing loss DFNA28 (Peters et al., 2002). Significantly, a genome-wide association study suggests that *TFCP2L3* gene variants are associated with age-related hearing impairment in European populations (Van Laer et al., 2008). In the inner ear *Tfcp2l3* shows a broad epithelial expression pattern by in situ hybridization. Finally, mutations in *ESRRB*, a member of the nuclear-hormone-receptor family of TFs, were linked with autosomal recessive hearing impairment DFNB35 (Collin et al., 2008b). *ESRRB* encodes an estrogen-related receptor beta (NR3B2), characterized by two functional domains, a DNA-binding domain and a ligand-binding domain. In the cochlea, it is specifically expressed in the endolymph-secreting marginal cells of the stria vascularis, controlling the expression of multiple ion channels essential for endolymph production (Chen and Nathans, 2007). Most prominent, the work on different *Esrrb* mutants mice reveals its function in controlling epithelial cell fate and fluid homeostasis in the inner ear.

Since the discovery of microRNAs, their essential role in post-transcriptional regulation of gene expression has been established in diverse processes including development, cellular signaling pathways, and disease (Kloosterman and Plasterk, 2006). MicroRNAs are short RNA molecules of approximately 21–23 nucleotides that mediate the posttranscriptional repression of mRNA of protein-coding genes (Bartel, 2009). A critical seed region within a microRNA is essential for its specific target recognition and thus mutations within this region are prone to alter its regulatory role. Recently, two complementary studies in humans and mice have revealed the first microRNA gene, miR-96, implicated in hearing loss. Mutations in the seed region of miR-96 leads to progressive hearing loss in humans underlying the DFNA50 locus (Mencía et al., 2009). As a result of this mutation, the biogenesis of miR-96 is disrupted and leads to a significant reduction of mRNA targeting. Similar to humans, an ENU-induced mutation of miR-96 in diminuendo (*Dmdo*) mice show progressive loss of hearing (Lewis et al., 2009). Further characterization of inner ear abnormalities of *Dmdo* mice include marked degeneration of cochlear hair cells and fused stereocilia of the remaining hair bundles. miR-96 belongs to a cluster of three microRNAs that includes miR-183 and miR-182, with all expressed in inner ear sensory cells, as well as in spiral ganglion cells. Transcription profiling of *Dmdo* mice has revealed altered expression of different genes, including downregulation of a prominent list of known deafness genes. Taken together, establishing the transcriptional and posttranscriptional regulatory networks of inner ear function may open a venue for future therapeutic strategies for some forms of hearing loss, utilizing modulation of gene expression and translation.

Fluid Homeostasis

Mechanosensory transduction of the auditory hair cells depends on two pivotal roles of the inner ear extracellular fluids. First, in response to sound stimulus, the conductive portion of the outer and middle ears transfer the energy of sound waves to the fluids within the closed compartment of the inner ear and triggers their movements. The hair bundles of the sensory hair cells are immersed within these fluids and are activated upon deflection as a result of their movement. Second, the unique chemical composition of the endolymph, with its high potassium (K^+) and low sodium (Na^+) concentrations, play a crucial role in hair cell depolarization. MET channels located within the tip of the stereocilia of the hair bundle are sensitive to mechanical movements that trigger their opening, allowing (K^+) influx into the hair cells. To support sensory transduction, a large group of genes encoding potassium channels and transporters, including

KCNQ1, *KCNE1*, *KCNQ4*, *KCNJ10*, and *SLC12A2*, participate in the critical mechanism of (K^+) recycling and its associated high endocochlear potential (reviewed in Zdebik et al., 2009). Mutations in these genes are associated with either NSHL or SHL of the affected individuals. A gap junction (GJ) network of several connexin (Cx) isoforms including *GJB2* (Cx26), *GJB6* (Cx31), *GJB6* (Cx30), are highly expressed in the ear and suggested to support the route of potassium recycling (Nickel and Forge, 2008). Human mutations in *GJB2* and *GJB6* underlie the most common forms of autosomal recessive deafness, DFNB1A and DFNB1B (del Castillo et al., 2002; Kelsell et al., 1997). Other connexins are involved in less frequent forms of hearing loss. Along with the pivotal role of potassium (K^+) ions in hearing, maintaining other electrolytes constituents, as well as controlling the volume of the extracellular fluids, is no less important for inner ear physiology and requires the function of additional proteins.

Mutations in the solute carrier transporter gene, *SLC26A4*, encoding pendrin, leads to prelingual deafness characterized by enlarged vestibular aqueduct (EVA). Some mutations can also lead to PS, a syndromic form of deafness associated with enlargement of the thyroid gland. Pendrin is expressed in different tissues including kidney, thyroid and inner ear, where it functions as a transporter of ions such as chloride (Cl^-), iodide (I^-), and bicarbonate (HCO_3^-). Functional assays have shown that different mutations along the pendrin sequence have different effects on its transport activity and thus can explain some of the clinical heterogeneity (Pera et al., 2008). In the inner ear, pendrin is localized to the membrane of different cell types that face the endolymph, both in the cochlear and vestibular apparatus. Whereas the number of pendrin transmembrane domains is still ambiguous in the literature (Dossena et al., 2009), it is clear that some of the mutations affect pendrin subcellular localization in a way that it fails to reach the plasma membrane (Brownstein et al., 2008). Generation of pendrin null mice has provided a tremendous tool in understanding the pathophysiology underlying this form of deafness observed in humans. *Slc26a4*$^{-/-}$ mice show a dramatic enlargement of the endolymphatic compartments with significant hydrops of the cochlea, as well as the endolymphatic sac and duct (Everett et al., 2001). Electrophysiological studies have shown that absence of pendrin leads to acidification of the endolymph, suggesting that pendrin mediates bicarbonate (HCO_3^-) secretion in the inner ear that buffers the accumulation of protons (H^+) (Wangemann et al., 2007). A subsequent increase of endolymphatic calcium (Ca^{2+}) ion concentration in pendrin null mice is attributed to the lower pH level that inhibits the acid-sensitive TRPV5 and TRPV6 calcium channels (Nakaya et al., 2007). Pendrin null mice also failed to develop endocochlear potential and hearing due to the loss of *Kcnj10* protein expression after the age of postnatal day (P)10 (Wangemann et al., 2004). In the cochlea, *Kcnj10*, encoding a K^+ channel, is normally expressed in intermediate cells of the stria vascularis and is sufficient for generating an endocochlear potential. Since pendrin is expressed in different cell types of the cochlea, its mutation is suggested to have an indirect effect on *Kcnj10* expression. An observed increased level of oxidative stress in pendrin null mice impairs normal function of the stria vascularis with subsequent loss of *Kcnj10* protein expression and lack of endocochlear potential (Singh and Wangemann, 2008). Interestingly, *KCNJ10*, together with mutations of *SLC26A4*, leads to digenic hearing loss with enlarged vestibular aqueduct (Yang et al., 2009). Understanding that the pendrin mouse model fails to develop hearing due to the loss of the endocochlear potential defines window of opportunities for therapeutic approaches prior to the loss of KCNJ10 protein expression.

The pendin null mice also show numerous developmental defects of the inner ear, consistent with cochlear hypothyroidism (Wangemann et al., 2009). The importance of normal thyroid function in hearing development and function is well established and has been studied in several mouse models with thyroid defects (Knipper et al., 2000; Mustapha et al., 2009; Winter et al., 2006). Since hypothyroidism has been observed in PS patients, it raised the question whether this factor contributes to the development of hearing loss in these affected individuals. The pendrin null mice were shown to have normal thyroid gland morphology and histology (Everett et al., 2001), while biochemical tests confirm that this mouse model is systemically euthyroid (Wangemann et al., 2009). Nonetheless, it has been proposed that the enlargement of the scala media distorts reciprocal epithelial-mesenchymal interactions that can lead to local hypothyroidism in the inner ear of pendrin null mice that affect its proper development. Further understanding the contribution of systemic hypothyroidism in PS patients to their hearing loss may help to identify a partial treatment based on thyroid hormone replacement strategies in order to prevent some of the auditory deformation.

Recent work focused on the vestibular dysfunction of an ENU mouse mutant for pendrin, loop, demonstrated that impaired function of pendrin leads to the formation of giant calcium oxalate stones in the inner ear (Figure 4; Dror et al., 2010). This unique type of mineral accounts for more than 80% percent of all kidney stones and its composition in the inner ear was revealed for the first time. A significant number of transporters and channels are expressed in both the kidney and inner ear, including pendrin (Lang et al., 2007). Understanding the functional significance of these genes in one system can shed more light on its role in other systems and thus contribute to a wide systemic networking of gene function.

Junctional Complex and Tight Junctions

The inner ear and its membranous labyrinth is composed of a rich network of epithelial cells that contribute to the compartmentalization of two types of inner ear fluids, the K^+-rich endolymph and Na^+-rich perilymph. The high concentration of potassium (K^+) within the endolymph is crucial for depolarization of the sensory hair cells upon stimulation driven by sound. Due to their unique ionic composition, the mechanical barrier created by different epithelial cells is crucial to prevent leakage of ions between these fluid-filled compartments. Paramount to this function are the tight junctions proteins that contribute to the contact between neighboring cells, thus maintaining the tight junctional barrier surrounding the scala media (endolymph). Several genes are essential for the molecular composition of tight junctions in the cochlea, including *CLDN14*, *TRIC*, and *TJP2*, have been linked with different forms of NSHL. Mutations in *CLDN14* encoding the tight junction claudin-14 cause

autosomal recessive deafness DFNB29 (Wilcox et al., 2001). Additional members of the claudin family of proteins, claudin-11 and claudin-9, are essential for auditory function in mice (Gow et al., 2004; Kitajiri et al., 2004; Nakano et al., 2009) The multiprotein complex of tight junctions is composed of integral and peripheral membrane proteins (Tsukita et al., 2001). The claudin family consists of more than 24 members of integral membrane proteins with four transmembrane domains being the main structural components of intramembrane strands. Changes in claudin composition of a junction that vary between different epithelia define the selectivity of ions within the paracellular pathway. In addition to their function in barrier formation and adhesion, the integral membrane proteins regulate cell polarization and recruit signaling molecules that regulate different cell mechanisms such as proliferation, differentiation, and gene expression (Matter and Balda, 2003). In the inner ear, claudin-14 is expressed in the sensory epithelium of the organ of Corti, restricted to tight junctions of the reticular lamina, a mosaic of sensory hair cells and different supporting cells (Ben-Yosef et al., 2003). Claudin-14 is also expressed in the vestibular apparatus of the inner ear, in the kidney and liver, although none of these systems have been shown to be affected in claudin-14 deaf individuals. The auditory system may lack the compensatory components essential for the loss of claudin-14 function (Wilcox et al., 2001). Claudin-14 null mice develop normal endocochlear potentials but show rapid OHC degeneration followed by slower IHC loss observed during the first 3 weeks of life. Freeze-fracture replicas show preserved morphological structure of tight junction strands, suggesting that other claudins participate in the cohesion of the cochlear junctional complex. Thus, the elimination of one claudin from a tight junction complex may have a functional but not necessary structural effect on strand integrity. Expression of claudin-14 in MDCK epithelial cells was shown to increase paracellular resistance via selective discrimination against cations (Ben-Yosef et al., 2003). In the inner ear, claudin-14 has been suggested to have a functional significance in maintaining the paracellular barrier against cations in the cochlear sensory epithelium. The absence of claudin-14 increases the permeability of cation that results in elevated K^+ concentration in the spaces between the outer hair cells (space of Nuel). Exposure of the basolateral membranes to this toxic environment causes prolonged depolarization of the outer hair cells that may lead to cell death (Zenner et al., 1994).

Tricellulin, encoded by the *TRIC* gene, is another tight junction protein important for auditory function. In contrast to claudins that mainly maintain bi-cellular tight junctions, tricellulin is enriched at the junctions between three epithelial cells (Ikenouchi et al., 2005). Several human mutations in *TRIC* have been linked with moderate-to-profound NSHL DFNB49 (Riazuddin et al., 2006). The *DFNB49* locus was originally mapped to a chromosomal region that also contains the *OCLN* gene encoding a tight junction protein known as occludin. Occludin is an integral membrane protein associated with the intramembrane claudin-based strands of the junctional complex (Feldman et al., 2005; Furuse et al., 1993). Similar to claudins, occludin has four transmembrane domains and was linked to various junctional functions, including regulation of size-selective diffusion of paracellular permeability (Balda et al., 2000). Utilizing carboxy and aminoterminal cytoplasmic domains, both claudins and occludin interact with a meshwork of cytoplasmic densely packed peripheral proteins that stabilize the junctional complex and serve as a scaffolding and a signaling center (Guillemot et al., 2008). A prominent example for these interactions is demonstrated by the tight junction proteins (TJP), TJP1, TJP2, and TJP3 (also known as ZO-1, ZO-2, and ZO-3) that interacts with the cytoplasmic domains of different integral membrane proteins such as claudins (Itoh et al., 1999) and occludin (Li et al., 2005). Tricellulin shares a conserved domain with occludin in its C-terminal cytoplasmic domain (occludin-ELL domain) that mediates the interaction with Tjp1 (Riazuddin et al., 2006). This domain was shown to be affected in the *TRIC* mutation that causes deafness in humans (Riazuddin et al., 2006). Tjp proteins contain multiple domains for protein-protein interactions, including three PDZ domains and an SH3 domain, through which they interact with membrane proteins, cytoskeleton components and signaling molecules. In particular, Tjp1 was shown to directly interact with F-actin, forming a molecular bridge between integral membrane proteins such as claudins and tricellulin and the cytoskeleton (Fanning et al., 2002). In the inner ear, tricellulin is expressed in tricellular junctions between supporting and hair cells. Human mutations in *TRIC* lead to a truncated form of tricellulin protein that lacks the functional domain (occludin-ELL), fails to interact with Tjp1, and leads to deafness (Riazuddin et al., 2006). It has been suggested that this mutation interferes with the ability to connect cytoskeletal actin of the hair cell cuticular plate to its associated stereocilia rootlets. Such structural deformation may affect the rigidity of the reticular lamina of the organ of Corti, leading to possible defects in stereocilia microdeflections, as well as structural aberrations of the sensory epithelium associated with mechanical stress of auditory function.

Recently, a mutation in a different member of the TJP proteins, TJP2 encoded by the *TJP2* gene, was linked with progressive NSHL DFNA51 (Walsh et al., 2010a). A tandem inverted genomic duplication that includes the entire wild-type *TJP2* gene leads to overexpression of both transcript and protein levels within lymphoblasts derived from affected individuals. Interestingly, the *TJP2* duplication affects the expression of apoptosis-related genes in the lymphoblasts. In the inner ear, Tjp2 was shown to be expressed in tight junctions between hair cells and supporting cells of the organ of Corti. Similar to other TJP proteins, Tjp2 mediates a wide spectrum of cellular signaling via its multiple protein-protein interaction domains (Guillemot et al., 2008). Tjp2 has also been reported to localize to the nucleus, where it interacts with several transcription factors, as well as with DNA-binding protein scaffold attachment factor B (SAFB) (Huerta et al., 2007; Traweger et al., 2003). It has been suggested that increased expression of TJP2 in the cochlear sensory epithelium of affected individuals alters the native intracellular signaling mediated by its normal function (Walsh et al., 2010a). As a result, deleterious changes in apoptosis-related gene expression in the sensory cells may lead to hair cell death and hearing loss.

Synaptic Transmission and the Auditory Pathway

Most forms of hearing loss interfere with the normal function of the cochlear sensory hair cells, which fail to transduce the

mechanical stimulus. However, in some cases, the hair cells are properly activated by sound but the electrical signal transmission from the cochlea to the brain along the auditory pathway is impaired. A relatively rare hearing disorder, coined by the nosological term auditory neuropathy, is caused by the improper function of the auditory nerve, while the cochlea sensory machinery is normal (Starr et al., 1996). Clinically, patients with auditory neuropathy show a significantly higher degree of speech disability as compared to patients with sensorineural hearing impairment with similar hearing thresholds (Starr et al., 2000). The clinical diagnostic criteria for auditory neuropathy includes abnormal auditory brainstem response (ABR) test and preserved otoacoustic emission (OAE), an indication of functional OHCs. Four genes have been discovered in association with auditory neuropathy. The role of the proteins encoded by these genes is well established for VGLUT3 (*SLC17A8)*, partly known for otoferlin (*OTOF*), and yet to be determined for pejvakin (*PJVK*) and diaphanous-3 (*DIAPH3*).

The primary lesion in auditory neuropathy patients can be located in the hair cells, the adjacent nerve terminals, the auditory nerve, the intervening synapses, or any other location further downstream of the auditory pathway. Interestingly, both VGLUT3 and otoferlin are key components of the afferent synapse of cochlear inner hair cells, which is the first synapse in the auditory pathway. VGLUT3 belongs to a group of three subtypes of the vesicular glutamate transporter (VGLUT 1–3). Glutamate is the principal excitatory neurotransmitter at the inner hair cell afferent synapse, as well as in the mammalian central nervous system (CNS) (Puel, 1995; Santos et al., 2009). The accumulation of glutamate in synaptic vesicles depends on the activity of the different VGLUTs isoforms. The role of the unconventional vesicular glutamate transporter VGLUT3 in synaptic transmission of the inner hair cell was first established in knockout mice (Seal et al., 2008). This work led to the identification of the *SLC17A8* gene, which encodes VGLUT3 and underlies NSHL (Ruel et al., 2008). The affected amino acid residue of VGLUT3 in DFNA25 individuals is highly conserved among all VGLUT isoforms, suggesting its functional role. *Vglut3* null mice show no response to sound measured by auditory brainstem response but preserved OAE indicates normal OHC function (Seal et al., 2008). Significantly, in the inner ear, Vglut3 is selectively expressed in cochlear IHCs. The synaptic vesicle of IHCs is loaded with glutamate mediated by the Vglut3 transporter. Accumulated glutamate synaptic vesicles then fuse with the plasma membrane, releasing their contents onto receptors of auditory-nerve terminals. Depletion of Vglut3 protein leads to a severe auditory synaptic deficiency due to the loss of glutamate uptake and release.

Otoferlin, encoded by *OTOF*, participates in the late step of synaptic-vesicle exocytosis in cochlear IHCs and has also been linked with auditory neuropathy (DFNB9) (Chaïb et al., 1996; Yasunaga et al., 1999). Otoferlin is a membrane-anchored cytosolic protein present as both long and short isoform classes (Yasunaga et al., 2000). All otoferlin isoforms share a C-terminal transmembrane (TM) domain, while they vary in the number of conserved C2 domains. The long isoforms were detected in both human and mouse, whereas the short isoforms were observed only in humans. The long isoforms contain six C2 domains (C2A–F), whereas the short ones contain two C2 domains. Interestingly, in all *DFNB9* mutations the long isoform is affected and is therefore required for normal auditory function in humans (Yasunaga et al., 2000). Otoferlin is a FER-1-like protein, a human homolog of the spermatogenesis factor FER-1, first described in *C. elegans* (Achanzar and Ward, 1997). Via its C2 domains, FER-1 mediates Ca^{2+} dependent lipid-processing events, crucial for vesicle fusion and regulated exocytosis (Washington and Ward, 2006). Prominently, the majority of the *DFNB9* mutations in otoferlin are distributed within the C2 domain, pointing out the functional necessity of this domain. Targeted mutagenesis of the C2 domains of FER-1 by single amino acid substitutions leads to defective membranous organelle fusion. Otoferlin also shows sequence similarity with dysferlin, another human homolog of FER-1, essential for vesicle fusion in muscle. Human mutations in dysferlin are associated with limb girdle muscular dystrophy and Miyoshi myopathy (Bashir et al., 1998; Liu et al., 1998). Based on well-established protein homology, otoferlin was proposed to mediate Ca^{2+}-triggered vesicle membrane fusions in IHCs. Further studies in this direction established the role of otoferlin as a unique calcium sensor for neurotransmitter release at the site of the auditory ribbon synapse (Roux et al., 2006). Detailed immunolocalization analysis showed that otoferlin expression is restricted to the IHC, with strong labeling at the baso-lateral synaptic region. Association of otoferlin with synaptic vesicles was further corroborated by its interactions with syntaxin1 and SNAP25, two members of the SNARE complex, as well as with the Cav1.3 calcium channel (Ramakrishnan et al., 2009; Safieddine and Wenthold, 1999). The interactions of otoferlin with these proteins were shown to be Ca^{2+} dependent, mediated by the C2 Ca^{2+} binding domains. Otoferlin-deficient mice lacking *Otof* exons 14 and 15, which encode most of its C2C domain, are profoundly deaf (Roux et al., 2006). Strikingly, $Otof^{-/-}$ mice show no visible ABR but have normal recordings of OAEs, mimicking the human phenotype and confirming auditory neuropathy. Importantly, ABR could be elicited by direct electrical stimuli, indicating that auditory neurons are not the target cells of the otoferlin deficit. Furthermore, transmission electron microscopy of $Otof^{-/-}$ ribbon synapses showed normal development, suggesting that otoferlin is not essential for their formation. However, electrophysiological studies of presynaptic function of IHCs revealed a severe disruption of Ca^{2+}-triggered exocytosis in the absence of otoferlin. Additional work has further established the critical function of otoferlin in calcium-dependent exocytosis and vesicle fusion at auditory hair cells synapses (Beurg et al., 2008; Dulon et al., 2009).

Recent work on *pachanga*, an additional mouse model for DFNB9, has yielded valuable insights into the molecular function of otoferlin. Originating in an ENU mutagenesis screen, the *pachanga* deaf mice, $Otof^{Pga/Pga}$, carry a missense mutation in the *Otof* gene, affecting an Asp residue in the C2F domain (Schwander et al., 2007). Significantly, as previously reported for synaptotagmin, Asp residues in C2 domains are essential for Ca^{2+} binding (Sutton et al., 1995). As opposed to the $Otof^{-/-}$ mice that lack otoferlin expression, $Otof^{Pga/Pga}$ mice provide an opportunity to study its function in the presence of the aberrant protein. Immunohistochemistry confirmed that $Otof^{Pga/Pga}$ mice

do express otoferlin in IHCs but to a lesser extent than control mice. Similar to the otoferlin null mice, *Otof*$^{Pga/Pga}$ show ABR thresholds above 90 dB with unaffected OAE, confirming an auditory neuropathy phenotype and normal function of OHCs. Electrocochleography studies of cochlear function indicated normal IHC MET and basolateral conductance (Pangrsic et al., 2010). Nevertheless, the absence of compound action potentials in response to loud acoustic stimulus narrows down the auditory defect to the IHC synapse. In vitro studies show that several characteristics of synaptic activity remain normal in the mutants, including vesicle docking, Ca^{2+} signaling, and fusion. However, *Otof*$^{Pga/Pga}$ mice show a significant slowed replenishment of the readily releasable vesicle pool (RRP). Such a lower rate of replenished vesicles within the mutant active zone limits the rate of transmitter release and reduces the neuronal spiking in response to sound stimulus. Altogether, the variety of studies on human and *Otof* mouse models revealed two functions of otoferlin in vesicle fusion at ribbon synapses (Beurg et al., 2008; Dulon et al., 2009) and in vesicle replenishment (Pangrsic et al., 2010). The impaired function of these processes in auditory synaptic transmission reveal the pathophysiology underlying hearing defects of *OTOF* affected individuals.

Mutations in two additional genes, *PJVK* and *DIAPH3*, have also been linked with auditory neuropathy. *PJVK*, encoding pejvakin, was the second gene discovered to be responsible for nonsyndromic auditory neuropathy (DFNB59) (Delmaghani et al., 2006). Pejvakin is a 352-residue protein that bears significant similarity to DFNA5, another deafness gene (Van Laer et al., 1998). It has been shown that the *DFNA5* gene has a potential role in the p53-regulated cellular response to DNA damage (Masuda et al., 2006) and has been identified as a target for methylation during cancer-related epigenetic events, highlighting its tumor suppressive activity (Kim et al., 2008). In the inner ear, pejvakin is expressed in different cell types, including the spiral ganglion, the sensory area of the vestibule and in portions of the organ of Corti. Further expression analysis along the afferent auditory pathway revealed that pejvakin is localized to the cochlear nuclei, superior olivary complex and inferior colliculus (Delmaghani et al., 2006). A knockin mouse model, *Dfnb59*^{tm1Ugds}, containing the human gene targeted by homologous recombination, show an increase in ABR thresholds with normal recording of OAEs at the affected frequencies, confirming these mice as a model for DFNB59 auditory neuropathy. *Dfnb59*^{tm1Ugds} mice did not show any gross malformation of the auditory system or along the auditory pathway. Excluding neuronal morphological defects such as demyelination suggested that the *Dfnb59* mutation is functional rather than structural. The identification of a novel mouse model, *sirtaki*, identified in a recessive ENU screen, demonstrated that pejvakin has more diverse mechanisms of pathogenesis (Schwander et al., 2007). *Sirtaki* mice have a premature stop codon, leading to a deletion of 62 amino acids of the pejvakin protein. Interestingly, unlike the neuropathy phenotype of *Dfnb59*^{tm1Ugds} mice, the *sirtaki* allele leads to progressive hearing loss in mice. Further OAE testing on *sirtaki* mice showed defects in OHCs, suggesting that pejvakin is necessary for their development and function. A different genetic background of these mice, as well as the different mutation in the *Pjvk* gene, can explain some of this phenotypic variation. Consistent with this observation, identification of a novel *PJVK* null mutation in a consanguineous Iranian family was shown to be associated with progressive hearing loss (Hashemzadeh Chaleshtori et al., 2007; Schwander et al., 2007). Correlating with the allelic variation in humans, the different phenotypic characteristics of the *sirtaki* allele, distinctive from other pejvakin mouse models, suggests that pejvakin affects both hair cells and neurons of auditory machinery.

Finally, the *Diaphanous homolog 3* (*DIAPH3*), was recently added to the list of auditory neuropathy-related genes. The *DIAPH3* gene originally mapped to the *AUNA1* (auditory neuropathy, dominant, 1) locus in an American family (Kim et al., 2004). Interestingly, as opposed to other auditory neuropathy genes that bear mutations within the coding regions (Delmaghani et al., 2006; Yasunaga et al., 1999), sequencing of all exons of *DIAPH3* revealed a mutation within the 5′ untranslated region (UTR). This mutation was detected within a GC box consensus sequence upstream to the translation initiation point of the mature mRNA. Several transcription factors belonging to the Sp1 and Krüppel-like protein families are known to bind to this highly conserved GC element, functioning as either repressors or activators of the translation machinery (Kaczynski et al., 2003). Analysis of lymphoblastoid cells isolated from affected individuals revealed 2- to 3-fold higher *DIAPH3* mRNA expression, detected by genome-wide expression arrays and quantitative RT-PCR (Schoen et al., 2010). Therefore, the *DIAPH3* mutation was proposed to have a gain of function effect among *AUNA1* affected individuals (Schoen et al., 2010). Furthermore, expression of constitutively active form of diaphanous protein in *Drosophila* leads to impaired response to sound by the Johnston's organ, JO. Future characterization of additional animal models will help to elucidate the possible role of *DIAPH3* in synapses or neurons of the auditory pathway expanding the understanding of its underlying molecular mechanisms.

Current Implications for Diagnostics and Therapy

Worldwide studies of the large variety of deafness genes are emerging rapidly. The most obvious and applicable clinical implication for gene discovery is diagnosis. Although the benefit of genetics studies for therapeutic approaches is still limited, in some cases genetics diagnosis is valuable. Auditory neuropathy provides a good example in this respect. Cochlear implantation among children with auditory neuropathy shows mixed results (Mason et al., 2003; Teagle et al., 2010). This should come as no surprise, since cochlear implants bypass the cochlear sensory cells but are limited when the lesion is further upstream of the afferent auditory pathway. Strikingly, cochlear implantation among DFNB9 patients with auditory neuropathy as a result of *OTOF* mutations is consistently successful as compared to other auditory neuropathies (Rodríguez-Ballesteros et al., 2003). The comprehensive understanding of *OTOF* pathophysiology in ribbon synapses of IHCs correlates with the successful cochlear implantations that bypass the IHCs, delivering electrical signals directly to the intact auditory nerve fibers. Such focused insight about the defective lesion along the auditory pathway based on genetics studies has provided clinics with the advantage to predict the efficacy of different therapeutic approaches, such as determining compatibility for cochlear implants.

Genetics studies in the field of hearing and deafness have also contributed to the prevention of some forms of hearing loss. Mutations in the mitochondrial 12S rRNA have been shown to be associated with high risk for aminoglycoside-induced hearing loss (Fischel-Ghodsian, 1999). Pharmacogenomic testing for such mutations is currently minimizing the risk for aminoglycoside-induced ototoxicity among groups of patients that are commonly treated with this drug (Bardien et al., 2009; Veenstra et al., 2007). Understanding the molecular pathogenesis associated with drug susceptibility of such genetic mutations can help promote the development of alternative drugs with similar efficacy yet significant reduced toxicity (Nudelman et al., 2009).

Looking toward the Future

The "holy grail" of auditory genetic studies is to use the enormous knowledge gathered about genes for hearing impairment and their function and apply it to advancing therapeutics for hearing. Whereas current gene therapy approaches are under development in animal models and in some human diseases, for example, Leber's congenital amaurosis, an eye disease in humans (Maguire et al., 2009), it is far from being applicable in the inner ear in humans. Several strategies are under development, taking advantage of high-throughput platforms and models for the human inner ear. One such strategy is wide screening of drug compounds for hearing loss using the zebrafish lateral line (Ou et al., 2009, 2010). This powerful high-throughput screening has led to the discovery of several compounds that protect the sensory cells from aminoglycoside-induced death. Moreover, this technique enables routine testing of the toxicity and efficacy of new drugs on the auditory system. Such an innovative approach may eventually enable the development of a protective medication for hearing loss, tailored to the specific genetic mutation of an individual patient.

Therapeutics in the inner ear field has focused on the regeneration capacity of hair cells (reviewed in Collado et al., 2008). The ability of avians to regenerate hair cells (Corwin and Cotanche, 1988; Ryals and Rubel, 1988) has triggered scientists to study the unique features that enable this capacity (Stone and Cotanche, 2007). Ultimately, revealing the mechanism of hair cell regeneration, not yet optimal in vertebrates, may pave the way to rescue hearing in hearing impaired patients. Recent advances in hearing therapeutics have also taken advantage of the stem cell field. Utilizing the pluripotency characteristics of embryonic stem cells, laboratories have devoted their efforts to differentiating these cells into hair cell-like structures (Coleman et al., 2007; Oshima et al., 2010). Ideally, hair cells derived from embryonic stem cells will be tested to recapitulate the morphological and functional properties of the cochlear hair cell, initiating stem cell-based treatment for hearing impairment. Another feasible approach is to enhance the capabilities of cochlear implants, a prosthesis that directly stimulates the nerve fibers of the cochlea, a current rehabilitation option for the population with severe to profound hearing loss (Shibata and Raphael, 2010). These efforts include, most recently, injection of adenoviral vectors with neurotrophins in order to generate resprouting of auditory peripheral fibers following hearing loss, which are required for the implants to electrically stimulate spiral ganglion neurons (Wise et al., 2010). Altogether, these emerging strategies are promising and hopefully will converge into combined future therapeutic approaches for deafness and hearing disorders.

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